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THE EXPERIMENTAL STUDY OF GENETIC RELATIONSHIPS¹

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The earliest experiment designed to determine the genetic relationships of a plant was carried out by Linnaeus and recorded in his famous disquisition on the sex of plants. He tells us that late in the autumn of 1757 he stumbled upon some plants of *Tragopogon hybridus* in the botanic garden at Upsala, growing in a bed where only *Tragopogon pratensis* and *Tragopogon porrifolius* had been planted. Before this time, as we know from Hartmann's dissertation on hybrid plants, Linnaeus had thought it possible that *Tragopogon hybridus* was a cross between *Tragopogon porrifolius* and *Lapsana stellata*—species of two quite unrelated genera. Now, however, he had a clue to its true relationship, which he set about to prove. The next year he removed the pollen from some heads of the yellow-flowered *Tragopogon pratensis* and sprinkled the styles with pollen from the purple-flowered *T. porrifolius*. The hybrid seeds thus obtained were planted in the fall and gave plants which were found to be identical with *Tragopogon hybridus*. The flowers were not completely purple, as in the staminate parent, but showed the influence of the pistillate parent in their yellow bases. After this experiment, Linnaeus tells us, it was impossible to doubt that new species might come into being by hybridization. His conception had changed greatly since the publication in *Fundamenta Botanica* (1735) of the oft-quoted dictum, "*Species tot numeramus, quot diversae formae in principio sunt creatae.*" (Every species which we can enumerate was created in the beginning a distinct form.)

The more mature views of Linnaeus, however, were in advance of his time. *Philosophia Botanica* (1737), in which the doctrine of special creation was set forth, continued, long after his death, to be the *vade mecum* of botanists. The conception of the hybrid origin of species was so completely disregarded that Sir James Edward Smith, an ardent Linnaean, the possessor of the Linnaean herbarium and

¹ Invitation paper read at the symposium on "The Genetic Relationship of Organisms" before the Botanical Society of America at Philadelphia, December 30, 1914.

founder of the Linnaean Society, wrote in 1807, "By species are understood so many individuals, or, among the generality of animals, so many pairs, as are presumed to have been formed at the creation, and have been perpetuated ever since; for though some animals appear to have been exterminated, we have no reason to suspect any new species has been produced; neither have we any cause to suppose any species of plant has been lost, or any new one permanently established, since their first formation, notwithstanding the speculations of some philosophers."

Although Linnaeus had reached the remarkable conclusion that species were, in some way, genetically interrelated, the second edition of the "Species Plantarum" and the inaugural dissertation of his pupil, Hartmann, contain so many preposterous guesses as to the parentage of certain supposed hybrid species, that he could certainly have had no conception of the limits of hybridization, or of the segregation which so often follows it. Obviously, he supposed that species originated as stable first generation hybrids—not a surprising theory, considering that, in his experiments with *Tragopogon*, he had chanced upon the one family in which parthenogenesis is so common that hybrids are often constant from generation to generation.

Linnaeus, knowing nothing about the geological history of organisms, had to construct a scheme of evolution which involved only the Mosaic "days of creation" and the few thousand years of recorded history. Having assumed the hybrid origin of species, it was clearly necessary for him to hypothecate the creation in the beginning of a considerable number of distinct forms representing the natural orders, for otherwise there could have been no material for hybridization to work upon. Moreover, since these originally created forms were so different that one could hardly believe in their crossing, he invoked miraculous intervention to account for it. Finally, in order to preserve intact the limits of the orders, he seems to have implied that the more fundamental characters were transmitted by the mother; the superficial ones by the father. His philosophical difficulties and the way he got around them now seem very amusing; but, as we shall see later, the same difficulties still confront certain modern geneticists, who have either not been able to circumvent them at all or are beginning to whisper a hypothesis more fantastic than that of Linnaeus.

Although Sir James Edward Smith's views were those which prevailed, for the most part, until the time of Darwin, there were occasional

outcroppings of evolutionary ideas. On the botanical side, the views of Dean Herbert and of Naudin were based largely upon experimentation rather than speculation and were expressed with especial clearness. The former (1822 and 1837) said that "horticultural experiments have established beyond the possibility of refutation, that botanical species are only a higher and more permanent class of varieties." The latter (1852) said that the methods by which the gardener produces new varieties coincide with the processes by which new species originate in nature. It remained for Darwin, however, to refute finally the doctrine of special creation.

Darwin ascribed the origin of new species in nature to the natural selection of favorable variations. With this view, in its most general aspect, nearly everyone is in accord. But the great problem of evolution still remains,—what is the source of the variations which are selected? Darwin could not answer this problem satisfactorily because no one had yet discovered the distinction between fluctuating variations, which are not inherited, and mutations, or germinal variations, which are inherited. He also went astray, as we now think, in believing that the effect of use or disuse of an organ could in some way impress itself upon the germ-plasm and become hereditary. The few who still hold that the selection of continuous variations would suffice to bring about specific differentiation can bring forward little or no evidence to support their view. The evidence all points to the utmost fixity of organisms, aside from mutations. In order not to perpetuate a misrepresentation of Darwin's views which he himself particularly resented, it should be said that, after the publication of the *Origin of Species*, Darwin came to believe that he had formerly underrated the value of mutations ("spontaneous variations") in bringing about diversity. Even in the first edition of his great work he stated his belief that the selection of insensible derivations had not been the exclusive means of modification. Darwin's caution, however, was not shared by all of his followers. Exaggeration and misrepresentation of his views led to an almost universal conviction that modification was too slow a process to be made the subject of experimental inquiry. Thus it came about that the *Origin of Species* was followed by a period of stagnation, as far as experimentation was concerned.

During this period there were indeed a few experimenters, with the courage of their convictions, who carried on genetical studies. One of them was Mendel, whose investigations aroused no interest among

his contemporaries, although they were stated with unsurpassed clearness, and would have changed the current of biological thought if anyone had realized their bearing on the theory of evolution. Another was Focke, who laid emphasis upon the importance of hybridization in species formation. Even then, as now, he believed that, in such large and polymorphic genera as *Rubus* and *Rosa*, many of the species have originated by hybridization, followed by the sorting out of stable forms.

From Darwin's time until very recently, however, it has been the prevailing view that the selection of extremes from a continuously varying population would result in a continuous, gradual modification of the entire population, and that such selection had brought about the formation of varieties in cultivation and of species in nature by the simultaneous transformation of masses of individuals. It need only be said that carefully planned and executed experiments lend no support to this view. All the evidence, on the contrary, seems to show that no amount of selection will suffice to modify the range of fluctuating variation of an organism. On the botanical side there is little evidence of the efficacy of selection as a factor in evolution; on the zoölogical side there are certain selection experiments of Castle's, carried out with characteristic care and accuracy, but surely capable of a different explanation from that which he gives. Even if correctly interpreted they have at best a dubious bearing on the problem of species formation. His experiments deal with the inheritance of a certain color pattern in rats, which, in a presumably homozygous race, may be modified in either direction by selection. The changes from generation to generation are very slight, however, and we cannot conceive of any agency in nature which would bring about assortive mating among such slightly dissimilar individuals. While it is becoming increasingly clear that the old selection theory is untenable, we are becoming more and more convinced that evolution does take place with measurable rapidity, and that the factors concerned with it are mutation and hybridization.

The new point of view we owe primarily to de Vries, who has determined the distinction between non-heritable fluctuating variations and inheritable germinal variations, or mutations, and has developed the mutation theory. It should appeal especially strongly to systematists, most of whom have really never been convinced of the adequacy of the discarded selection hypothesis. Why, if species had come about by

such a gradual process, were not intergradations more common? Why did most of the supposed intergradations prove to be rare and partially sterile hybrids? Why, if evolution were still going on, could one recognize speciation at all in some of the groups? To the theoretical evolutionist, who knew as little as possible about species, such questions indicated merely the perversity of the systematist. The latter, accepting the general truth of evolution, but influenced hardly at all in his attitude by the manner in which it was supposed to have taken place, continued to describe species and then still more species, just as he had always done, and just as he will doubtless continue to do. Sometimes he told what he thought about their relationships, oftener he did not, but he seldom failed to add to his description some variation of the formula "entirely distinct from its nearest ally." He has been anathematized; some of his colleagues have even threatened to cast him into outer darkness. Nevertheless, his work has certainly been as truthful and as serviceable as the work of those who deplore his "raking together of straws and sticks and even antique dust."

It will require the combined efforts of morphologist, systematist, and geneticist to arrive at the whole truth in regard to genetic relationships. In one way the geneticist has a great advantage over the other workers, for his methods are inductive, whereas theirs are deductive. In another way he is at a great disadvantage, for he can deal only with the lesser categories, the variety, the species and perhaps the genus. The relationship of the larger groups must be determined, if at all, by the deductive methods of the morphologist, and, I may add, of the biochemist; for, as the years go on, biochemistry will come to be applied more and more to the elucidation of genetical problems.

The immediate aims of the geneticist are (1) to observe the origin of new and distinct forms, the genetic relationship of which must, therefore, of necessity be known, (2) to determine the conditions which brought these forms about, and (3) to study their hereditary behavior and their morphological and chemical characteristics in order to provide a basis for sound deduction in regard to multitudes of types which we can never hope to know except as facts of nature. All three aims have already been realized in some measure as a result of the recent activity in genetics.

Most of the new forms of which the origin has been observed, belong to one class of organisms—namely, recessive Mendelian varieties. Such varieties have been observed to originate in two ways,

by mutation, and by segregation from hybrids. Certain ultra-Mendelians maintain that only the latter method is operative, but their argument, which will be discussed after certain examples have been called to mind, can be supported by little or no evidence. As examples of recessive mutations, one naturally thinks first of the remarkable series of 150 which Morgan has observed to originate from the fruit fly, *Drosophila ampelophila*. All of them are recessive to the typical form. On the botanical side there is no correspondingly complex series of recessives known, of which all the members have originated under scientifically controlled conditions, but there is hardly the least doubt that the cases of the sweet pea (*Lathyrus odoratus*), of *Primula obconica* and of *Primula sinensis* are quite comparable with that of *Drosophila*. Some members of each of these large series of forms are known to have come about as mutations, and probably all did. Bateson, who has devoted especial attention to the sweet pea, is sure that all the varieties are recessive to the wild prototype, *Lathyrus odoratus*. It seems sufficiently well proved that recent hybridization has not modified *Lathyrus odoratus*, for no other plant has been found which will cross with it.

In nature there are many examples of species and varieties which bear simple Mendelian relationship to one another. Such a case has been discovered by Trow in the series of elementary species comprehended under the name *Senecio vulgaris*. It also appears that in *Antirrhinum* there are species which seem to be very unlike, nevertheless all of the differences between them can be determined by Mendelian analysis. A large number of stable forms segregate from a hybrid between two such species, and these forms are themselves indistinguishable from species. Their fertility is unimpaired. None of them contains a single character which is not identical with, or recessive to, a corresponding character in one or the other of the parents. Baur and Lotsy have studied the hybrids of *Antirrhinum molle* and *A. majus* from a Mendelian standpoint. The latter has been so impressed by the results that he has come to believe that there is no source of variation in nature except hybridization followed by segregation; it seems to him the sole method of species formation. He explains the so-called recessive mutations, no matter how rare they may be, by the assumption of previous hybridization and a sufficiently large number of multiple factors. It seems fair to ask the holder of this view how the forms originate which supply the characters to be assembled and

reassorted by hybridization. This question brings him face to face with the philosophical difficulties of Linnaeus. Either he must deny evolution, or, with Bateson, confess that the studies carried on by Mendelians have thrown no light on the problems of evolution. Bateson suggests that we should seriously consider the possibility that evolution has not taken place from the simple to the complex, but rather from the complex to the simple; that the original forms of life were heterozygous with regard to all the characters which have ever appeared in geological history; that for each character there was a corresponding inhibitor, and that the characters have come successively to light by the segregation of recessives from which the inhibitors have fallen away. We cannot believe, from the tentative way in which Bateson proposes this fantastic hypothesis, that he really places much faith in it. But the mere fact that he should whisper it shows the extreme pessimism of the ultra-Mendelian attitude in regard to the problem of evolution. Other experimental workers, however, are more optimistic. The Mendelians have been so firmly convinced that differences between species were all capable of Mendelian analysis that they have disregarded facts which did not fit their formulations. Professor de Vries' work is set aside with the statement that the chief reason why factorial analysis has been declared to be inapplicable to the *Oenothera* mutations is because no one (except Heribert-Nilsson) has set about such an analysis in the right way.

Even those of us who doubt the universality of the Mendelian phenomena, see no reason to deny that species formation by hybridization and subsequent segregation has taken place on a large scale in many groups. One may, however, admit the great prevalence of hybridization, without believing that all variation which may take place subsequently to hybridization is a result of that hybridization. In other words, there is no reason why true mutations should not occur in hybrids as well as in pure lines.

In this connection we may examine a little more closely the view that recessives always originate by segregation rather than by mutation, a hypothesis ancillary to the multiple factor hypothesis. In only one case among wild plants has it been satisfactorily shown that a recessive mutation differs from the parent form in the lack of duplicate factors. This is the case of *Capsella Heegeri* and *Capsella Bursa-pastoris*, which has recently been studied by Shull. He finds that there is a difference of two duplicate factors between the derivative

and the parent form. The presence of either factor suffices to give the capsule the triangular shape typical of *Capsella*. *C. Heegeri*, the recessive, has a capsule shaped like that of *Camelina*, so that it really departs from the parent form in a generic character. As Solms-Laubach has shown, no systematist who did not know of its mutative origin or of its relationship as determined by crossing would place it in the genus *Capsella*. All the plants in existence have descended from one individual which was found by Professor Heeger in a colony of *Capsella Bursa-pastoris* in the market-place at Landau, Germany. When the two species are crossed the first hybrid generation is, of course, uniform and like *Capsella Bursa-pastoris*. In the second generation there is one plant of *C. Heegeri* in 16. The 15 sister plants all look alike, but are in reality of three kinds. In the third generation seven will remain constant, four will give one plant of *C. Heegeri* in 4, and four will give one plant of *C. Heegeri* in 16. As far as experimental evidence can be applied it shows that a plant into which the character of *C. Heegeri* has been introduced by hybridization must either give rise to *C. Heegeri* not less than once in 16 times, or not at all. Most recessives, if crossed with the parent form, reappear in the second hybrid generation in a typical 1 : 3 ratio. If they originated according to the multiple factor hypothesis we should have to assume that factors which had been independent for countless generations could suddenly become indissolubly associated in the generation in which the pure recessive appeared. There is not the least evidence that this takes place. Now that we have cases in which segregation in the second hybrid generation occurs in the ratios 15 : 1 and 63 : 1, we naturally expect that some of the recessives which are supposed to depend upon the concurrent absence of so many characters would not reappear at all when recrossed with their parents. With as few as six independent identical factors there would be only one recessive in a second hybrid generation consisting of 4,096 individuals. In a case like that of *Capsella Heegeri* it would be necessary to postulate more characters than six to account for a non-mutative appearance so seldom that only one individual has ever been observed. On the whole there seems no reason to doubt that the sporadic appearance of recessives in supposedly pure lines is really due to mutation. The imaginary influence of past hybridization is a bogey that need not bother us.

Although recessive mutations and recombinations following hybridization may contribute much to specific and varietal diversity,

it is obvious that they cannot bring about the origin of any fundamentally new type. We cannot, therefore, have any comprehensive knowledge of the genetic relationships of organisms until we know something about progressive mutation. Mendelian researches have shown us how to verify supposed relationships in the simplest cases, those in which characters have been lost. But can we always be sure that the recessive is the derived form? Is it not sometimes true that the dominant is the derived form, and that a new character has appeared? On this point the evidence is very unsatisfactory. Cases of progressive evolution have indeed been observed, but for the most part they have occurred out of the beaten track of the Mendelian and have been largely discounted, for Mendelism is the present fashion in genetics. In *Primula sinensis*, however, Professor Keeble has been fortunate enough to observe the origin of a Mendelian dominant.

There are two types of gigantism in *Primula*. In one type the chromosome number is the same as in the typical forms, and the giant acts as a simple Mendelian dominant when crossed with the parent. The other type is of a very different nature, and will be referred to later. A number of giants of the simple Mendelian type have been known among the cultivated varieties of *Primula*, but no one knew how they had originated until the "Giant White Queen Star" appeared as a mutation in the third guarded generation of a pure line of the "White Queen Star" variety. It came true from seed and has remained uniform through five successive generations, which included several hundred individuals. Although we cannot doubt in this case that a new dominant has arisen, there is unfortunately one flaw in the evidence. The new form has proved sterile when crossed with the parent form and with all other non-giant forms, so that its dominance is only inferred from its perfect analogy with other giants of the same type. The latter have been found by Gregory to show simple dominance over the non-giant forms. Our argument is based upon evidence fitted together from two sources. Other examples might be brought forward, but they are not as striking or as well authenticated as this one. There is a type of non-Mendelian progressive evolution, however, which has been far more satisfactorily studied. I refer to mutations in which new characters may be definitely associated with mutative changes in the chromosome number.

It appears that, in general, each species is characterized by a constant number of chromosomes. Within a genus, different species

are frequently characterized by different chromosome numbers; and, exceptionally, within a species, well-characterized nominal varieties may differ in this respect. Lists of genera in which there is variation in chromosome number have been published by Gates and by Strassburger. Among the genera included in these lists, or concerning which information has been more recently published, we find the flowering plants *Alchemilla*, *Antennaria*, *Crepis*, *Dahlia*, *Daphne*, *Drosera*, *Funckia*, *Hieracium*, *Humulus*, *Houttuynia*, *Musa*, *Oenothera*, *Primula*, *Rosa*, *Rumex*, *Spiranthes*, *Taraxacum*, *Thalictrum*, and *Wikstroemia*, the ferns *Athyrium*, *Lastrea* and *Nephrodium*, the mosses *Amblystegium*, *Bryum*, *Mnium* and *Phascum*. The numbers range from 3 (x) and 6 ($2x$) in *Canna* to 48 (x) and 96 ($2x$) in *Castalea*. The processes of species formation must have been frequently attended by changes in the number of chromosomes. We cannot escape this conclusion when we take into consideration the two striking facts that within a species the number is constant, but that from species to species and from genus to genus it shows the greatest diversity.

Recent discoveries are making it very clear that mutative changes in the chromosome number occur frequently, and that such changes are always associated with a modification in the morphological characters of the plant. In other words, certain mutations are probably dependent upon, or, at any rate, closely associated with, visible changes in the nuclear mechanism. We have every reason to believe, therefore, that the different chromosome numbers of different species were acquired simultaneously with the acquisition of other specific characters.

Among the mutations of *Oenothera Lamarckiana* there is just one which de Vries regards as unquestionably progressive. It is the remarkable *Oenothera gigas*, which differs from its parent not only in numerous external characters but also in having twice as many chromosomes. The gametophytic and sporophytic numbers are 14 and 28 in *Oe. gigas*, 7 and 14 in *Oe. Lamarckiana*. Gates has shown that the double chromosome number of the former is correlated with a larger cell size in all corresponding tissues and that many of the gross characters of the plant are in turn dependent upon the difference in the cells. Hybrids between *Oe. gigas* and *Oe. Lamarckiana* show no semblance of Mendelian inheritance. In general they are very sterile, but in one case a fertile strain was obtained which remained constant through five generations. The hybrids are intermediate whichever way the cross is made, and, if back crossed with either parent, the

secondary hybrids are again intermediate. Clearly we are not concerned here with a new dominant, in the Mendelian sense, but rather with the simultaneous origin of a whole group of non-Mendelizing characters, each of which is correlated with the increase in the number of chromosomes.

Among the flowering plants we do not know of any way in which the number of chromosomes may be experimentally modified. Among the mosses however, the brilliant work of the Belgian investigators Élie and Émile Marchal has shown that tetraploid races may be produced at will. Their results are of the greatest importance because of the light which they throw upon such spontaneous mutations as *Oenothera gigas*.

It has long been known that moss protonemata might be regenerated from bits of the seta or young capsule; in the past both Pringsheim and Correns have obtained such aposporous protonemata. The investigations of the Marchals were undertaken to determine (1) whether such protonemata would or would not give rise to moss plants with antheridia and archegonia, (2) whether, in case such plants were obtained, the gametes would have the $2x$ chromosome number of the normal sporophyte and give rise to a new sporophyte with the tetraploid chromosome number, and (3) whether the tetraploid races would be like the typical form of the species, or different. It was found that diploid gametophytes were obtained in which the vegetative cells were larger than in normal gametophytes and the generative cells were twice as large. The diploid gametophytes of dioecious mosses were absolutely sterile, and synoecious. They could be maintained in culture only by regeneration from pieces of the axis. In the case of the monoecious mosses the results were far more interesting, for the diploid gametophytes gave rise to tetraploid sporophytes, which produced good spores, and in turn reproduced the diploid gametophyte. The fertile races thus experimentally obtained were named *Amblystegium serpens bivalens* and *Amblystegium subtile bivalens*. Cytological studies showed that the reproduction of these new races was by normal fusion of the diploid gametes. The cytological relations were worked out for *Mnium*, *Bryum*, *Amblystegium* and *Phascum*. In the case of *Amblystegium* it was found possible to double the chromosome number again by regeneration from the tetraploid sporophyte, but the tetraploid gametophytes thus obtained were completely sterile. The new bivalent race obtained from

Phascum was anomalous in that it was dwarf, rather than giant. It was sterile, but reproduced itself by propagula, which do not usually occur in the parent form, *Phascum cuspidatum*. Many mosses reproduce almost exclusively by vegetative means, and this experiment is in the highest degree suggestive of the manner in which this habit has come about. All of the other bivalent races differed from their parents in characters which obviously depended upon the greater size of the individual cells.

The experiments of the Marchals give us the strongest reason to believe that the visible differences between *Oenothera Lamarckiana* and *Oe. gigas* have been correctly interpreted as due to the doubling in the latter of the chromosome number. This explanation, first proposed by Gates, has been assented to by de Vries. The latter points out, however, that the genetical and physiological qualities of *Oe. gigas* are entirely different from those of *Oe. Lamarckiana*. The seeds of *Oe. gigas*, for example, are much more viable than those of the parent species, and germinate more quickly. *Oe. gigas* does not give the *laeta* and *velutina* twin hybrids when crossed with unrelated species, as *Oe. Lamarckiana* does. Obviously, then, the process of mutation has changed the hereditary qualities of the germ plasm even more than the morphological characters of the sporophyte. *Oe. gigas* gives rise to certain secondary derivatives which have no counterparts among the variations of *Oe. Lamarckiana*. Are we too optimistic if we view the former as a newly evolved center about which an entirely new series of specific variations may spring up?

It is natural to ask at this point how commonly mutative changes in the chromosome number occur. It is known that in *Oe. lata*, one of the most characteristic of the mutations from *Oe. Lamarckiana*, the unreduced number is 15 instead of 14. In *semigigas* mutations from *Oe. Lamarckiana* and *Oe. biennis* the number is 21. I have recently investigated the mutability of several species of *Oenothera* which belong to the small-flowered, self-pollinating series of forms which are generally lumped together under the name "*Oe. biennis*." Two of them have given rise to mutations characterized by an increased chromosome number. The cytological investigation of these new mutations has been undertaken by Mr. E. G. Arzberger, through whose kindness I am able to announce that *Oe. stenomeres* mut. *gigas* has 28 chromosomes, and that *Oe. pratincola* mut. *gigas* also has this number. The former seems in every way analogous to *Oe. gigas* de Vries,

and will make it possible to determine the hereditary qualities of the latter much more satisfactorily than has thus far been possible. The method, of course, will be to compare the hybrids between the parent forms with those between the *gigas*-mutations. The crosses in both cases will be between gametes with the same number of chromosomes, so we need not expect the high degree of sterility which prevents the study of hybrids between the forms characterized respectively by 14 and 28 chromosomes. It would give increased significance to the discovery of the new *gigas*-mutations if tetraploid species of *Oenothera* should be found in nature. I mention this fact in the hope that everyone who is interested in the problem of mutation may be on the lookout for them. I already have one wild species in my garden which appears to possess certain traits of the *gigas*-forms but Mr. Arzberger was unable to get his preparations ready in time to make a chromosome count before this meeting took place.

It has already been mentioned that in the genus *Primula* two types of gigantism occur. One type is characterized by a doubling of the chromosome number and seems to represent the same type of mutation as that which in *Oenothera* gives rise to the *gigas*-form. Gregory has found that not only are the chromosomes doubled in the tetraploid *Primulas*, but also the Mendelian factors for numerous characters. The hereditary behavior of these mutations is, therefore, entirely different from that of the diploid races.

The differences in chromosome number which occur among species of the same genus represent changes which must necessarily have taken place abruptly. We can not imagine the origin of a *gigas*-race by gradual selection or by Mendelian segregation. Heribert-Nilsson has indeed offered a Mendelian explanation of *Oe. gigas*, but he has wholly neglected the cytological facts in the case. In *Oe. stenomeres* mut. *gigas* the chromosome count was made in the generation following the first appearance of the mutation. In the corresponding mutation from *Oe. pratincola* the count was made in the original mutation. There is the best of evidence, then, that the new chromosome number is acquired simultaneously with the new morphological characters. It is more reasonable to believe in a causal relationship between the cytological and morphological changes than to believe that the latter result from the hypothetical influence of hypothetical crossing in the indefinite past. It cannot be assumed that the modification of the chromosome number is itself due to Mendelian segregation, for there are too many facts

which go to show that the chromosomes are themselves the mechanism by which Mendelian characters are transmitted. It is only necessary to call to mind Gregory's discovery that the Mendelian factors are doubled in tetraploid plants of *Primula*. If the mechanism is altered, the genetic qualities of the plant must of necessity be altered. For this reason I am disposed to lay especial emphasis upon those mutations in which a cytological change in the cell can be demonstrated, not, however, without stating my belief that mutations for which there is no visible basis are quite as independent of ancestral hybridization as the *gigas*-mutations. In certain groups, as the *Coniferae* for example, there is great uniformity in the chromosome number, showing that evolution has taken place through invisible modifications of the germ plasm. Probably most of the mutations which take place in any group of plants, even *Oenothera*, have the same chromosome number as the parent.

My *Oenothera* cultures of the last three years have given many mutations, aside from the *gigas* forms, which confirm in all essential points de Vries's experience with *Oe. Larmarckiana*. Some of them I regard as progressive, although it has not been possible yet to demonstrate that they are dominant in a Mendelian sense. In fact, Mendelian inheritance seems to play so small a part in *Oenothera* that in general we cannot expect to apply the test of dominance in judging of the progressiveness of mutations. We must consider a mutation as progressive when it shows characters which are not present in the parent.

It has been shown by de Vries that reciprocal hybrids between *Oenothera* species are frequently very unlike one another. Both de Vries and Davis have encountered cases in which the hybrids are strongly patroclinic. I have just the opposite experience with some of my interspecific hybrids, which are strikingly matroclinic. It is clear that in this genus reciprocal hybrids may be either matroclinic or patroclinic. In either case it is impossible to say that the characters of one parent or the other are dominant in the ordinary sense. With this explanation I shall proceed to a very brief discussion of two of my new mutations.

In *Oenothera pratincola* the seedling leaves are ovate. Seven different strains of this species have given rise to a mutation with round seedling leaves, which I have called mut. *nummularia*. In three strains the mutation has appeared in three successive genera-

tions, with a frequency of approximately one mutation for every 400 seeds sown. Correlated with the shape of the leaves are other characters, involving the size and branching of the plant and the pubescence and dehiscence of the calyx. The entire group of characters are coherent; it may be predicted from the shape of the seedling leaves alone that the other characters will appear in the mature plant. Nevertheless it is quite impossible to imagine any necessary inter-dependence between the characters which cohere in this mutation. No systematist who did not know the parentage of mut. *nummularia* could possibly decide which of two dozen elementary species in my garden had given rise to it.

Reciprocal hybrids between the parent species and the mutation appear to be strictly matroclinic, but the plants are still very young seedlings. The progeny of the cross mut. *nummularia* \times *f. typica* are all mut. *nummularia*, conversely, the progeny of the reciprocal cross are all *f. typica*, except for the fact that mut. *nummularia* appears with its usual frequency of one plant in several hundred. It seems that only female gametes bear the group of characters which distinguish mut. *nummularia* from *f. typica*.

The other mutation which I wish to mention is *Oe. stenomeres* mut. *lasiopetala*. It differs from its parent species in a group of coherent characters, one of which is the hairiness of the petals. The solitary primary mutation when self-pollinated gave rise to a progeny consisting of typical *Oe. stenomeres* and mut. *lasiopetala* in a ratio suggesting 1 : 1, although the former was in excess. It is highly improbable that a Mendelian explanation will apply to this case, either, but I do wish to point out that such an explanation would necessitate viewing mut. *lasiopetala* as a dominant. A recessive could not have thrown the dominant parent.

As my experience with this highly interesting group of plants increases I am more and more convinced that de Vries' conception of the origin of species is the true one. He believes that new species, differing from the old ones in a coherent group of characters, may come into existence at one step, by mutation. The evidence for this special view of mutation has been doubted by several critics, who have brought forward several destructive arguments. I believe that all of these arguments can be met.

There is first the argument that *Oenothera Lamarckiana* is known only in horticulture, and may be a garden product; consequently that

its behavior cannot be held to throw light upon the behavior of wild plants. It has now been shown that wild species show absolutely parallel phenomena.

It has also been said that the non-Mendelian behavior of *Oenothera* hybrids shows that the genus is so exceptional in its genetic behavior that it provides no basis for generalization. In reply we may say that the Mendelian school have in general confined their crosses to varieties of cultivated plants. If they had ventured into the field of inter-specific hybridization they would have found plenty of parallels to the behavior of *Oenothera*. As a case in point we may cite the experience of W. Neilson Jones, who obtained matroclinic reciprocal hybrids between species of *Digitalis*. He did not think the results quite comparable to those of *Oenothera* for the reason that the *Oenothera* hybrids reported up to that time had been patroclinic. Now, however, this argument is removed by the discovery of matroclinic hybrids in *Oenothera*. The enormous literature of orchid hybridization contains frequent allusions to unlike reciprocal hybrids. In this largest family of the monocotyledons may be found numerous examples of both patroclinic and matroclinic hybrids. Many of them may prove to be cases of parthenogenesis, but the situation demands a much more thorough study than has yet been given it. The so-called "false hybrids" of *Fragaria*, as well as other Rosaceae, should be carefully investigated both cytologically and genetically. This much is sure; it is not yet time to speak of the universality of Mendelian phenomena, or of the exceptional nature of *Oenothera*. I am inclined to believe that such groups as the Orchidaceae may even provide parallels for the mutability of *Oenothera*. For example, Miss Pace has recently studied the cytology of *Spiranthes cernua* and *S. gracilis* from material collected near Chicago. Nine other species are interpolated between these two by Oakes Ames, in his Monograph of the American Species of *Spiranthes*. Yet they differ from one another in somewhat the same way that *Oenothera Lamarckiana* differs from *Oe. gigas*. Miss Pace finds 15 and 30 chromosomes in *Spiranthes gracilis*, as the reduced and unreduced numbers, but 30 and 60 in *S. cernua*. The latter may well be, as Miss Pace intimates, a tetraploid form of the former. Here is a fertile field for the experimentalist.

Jeffrey has lately argued that all plants which have any defective pollen grains are in a state of genetical impurity, and that any conclusions drawn from their genetical behavior, in connection with the

vexed problem of the origin of species, must be subject to a large degree of reserve. Since all of the mutant *Oenotheras* are characterized by more or less defective pollen, he thinks that the mutations are segregates from hybrids, and that "the mutation theory of de Vries appears accordingly to lag useless on the biological stage, and may apparently be now relegated to the limbo of discarded hypotheses." Fortunately for the mutation theory, Professor Jeffrey's argument is not sound. In the first place it must be insisted that there can be no such thing as a morphological test of genetic impurity. We can only recognize genetical impurity by genetical tests. There is a certain sterile variety of the sweet pea, which, according to Jeffrey's pollen test, would be adjudged a hybrid. When crossed with forms with normal pollen it acts as a simple recessive, and like the other recessive varieties of this plant it has doubtless arisen by mutation. Bateson, who has critically studied this series of varieties, writes of, ". . . the sweet pea, a form which is beyond suspicion of having been crossed with anything else, and has certainly produced all the multitude of types which we now possess by variations from one wild species." Again, he states that "in spite of repeated trials, no one has yet succeeded in crossing the sweet pea with any other leguminous species." In the sweet pea, then, we find pollen conditions identical with those which Jeffrey believes are found only in hybrids; nevertheless there is no reason to believe that hybridization has ever occurred in the species. We may turn to another case. *Humulus Lupulus*, the hop, is normally dioecious, but monoecious individuals occur now and then which can hardly be considered as other than mutations. Winge has recently studied the pollen of one of these monoecious plants, found wild and transplanted from the woods into his garden at Carlsberg, Denmark. It bore staminate inflorescences at the base of otherwise hop-bearing branches. Cytological study showed that pollen mother cells were formed which divided normally but thereafter shrivelled up without making the tetrad division. Winge himself points out the similarity between this case and that of *Oenothera lata*. Of all the mutations from *Oenothera Lamarckiana*, *Oe. lata* is the most sterile. It is generally completely so, but two or three strains are now known which yield a small amount of good pollen. Monoecious hop plants likewise vary greatly in pollen fertility, and Winge has lately made pollinations with apparently quite normal pollen from a monoecious plant. Winge worked with the wild hop of northern Europe. Aside from geographic

races of *Humulus Lupulus*, and the variations of it which have arisen in cultivation, there is no other form in the genus except *H. japonicus*. The former has 20 chromosomes in somatic cells, the latter 16. The cross *H. japonicus* \times *H. Lupulus* cannot be made; the reciprocal cross yielded variously malformed embryos, none of which were capable of further growth. So in this case again there can be no suspicion that the pollen sterility has come about through hybridization.

All plants which show transition from hermaphroditism to dioecism or monoecism present conditions parallel to those in *Humulus*. The case of *Plantago lanceolata* happens to be especially familiar to me. In most strains of this species the flowers are all perfect and the pollen is good. There are other strains, however, which are gynodioecious. Half of the plants are normal hermaphrodites and the other half functionally pistillate. I say functionally pistillate because in many of these strains the anthers develop, and contain pollen, but the grains are much smaller than normal pollen and are not liberated by the dehiscence of the anthers. Probably they are non-functional. From this condition there are various gradations through strains in which the anthers of the functionally pistillate form contain no pollen to strains in which the stamens are replaced by staminodia. In the latter form the gynodioecious state is not only functionally but also structurally attained. *Plantago lanceolata* is an introduced weed in the United States. In the Old World the well-marked subgenus to which it belongs contains six other species, but all are of comparatively restricted distribution. In northern Europe, as in the United States, where the sex forms have been studied, there is no allied species with which it could hybridize. We have, therefore, no reason to suspect that anther sterility in *Plantago lanceolata* has any relationship to hybridization. On the contrary, we may assume that the dioecious states of the species have been attained by a series of mutations, and that pollen conditions simulating those in hybrids may come about by mutation as well as by hybridization.

At the risk of being tiresome there is one more type of anther sterility which I wish to touch upon. Bateson says: "Without much more critical data I suppose no one would nowadays be inclined to follow Darwin in instituting a comparison between the sterility of hybrids and that of illegitimately raised plants of heterostyle species. It is even difficult to imagine any essential resemblance between these two phenomena, nor has evidence ever been produced to show that

illegitimately raised plants have bad pollen grains, which is the usual symptom of sterility in hybrid plants. . . ." This statement does not do justice to Darwin's evidence, which is quite convincing, especially in the case *Lythrum salicaria*. This species is trimorphic, *i. e.*, its flowers are long-styled, mid-styled or short-styled. Each flower type has two sets of stamens, coinciding in length with the styles of the two other types of flowers. Thus there are three kinds of stamens in the species and each bears pollen which is morphologically and physiologically different from that of the others. A pollination is legitimate when it takes place between a style and stamens of the same length. There are 18 possible combinations between the 3 forms, of which 6 are legitimate and 12 illegitimate. Darwin made all the different pollinations and found that only the legitimate ones were fully fertile. In regard to pollen sterility we will quote his own words. An illegitimate progeny from the long-styled form, pollinated from the longer or shorter stamens of the same form, consisted of 56 plants, belonging to three lots. "In several plants of all three lots, many of the anthers were either shrivelled or contained brown and tough, or pulpy matter, without any good pollen grains, and they never shed their contents; they were in the state designated by Gaertner as contabescens. . . . In one flower all the anthers were contabescens excepting two, which appeared to the naked eye sound; but under the microscope about two thirds of the pollen grains were seen to be small and shrivelled. In another plant, in which all the anthers appeared sound, many of the pollen-grains were shrivelled and of unequal sizes." An illegitimate progeny of nine plants resulted from the pollination of the short-styled form with pollen from the shorter stamens of the same form. "The anthers in many of the flowers on several plants were contabescens." Of 25 illegitimate plants from the mid-styled form, pollinated from the shorter stamens of the long-styled form, the pollen of 4 plants was examined "during the highly favorable season of 1866 . . . ; in one mid-styled plant, some of the anthers of the longer stamens were contabescens, but in the other anthers the pollen grains were mostly sound, as they were in all the anthers of the shorter stamens; in two other mid-styled and in one long-styled plant many of the pollen grains were small and shrivelled; and in the latter plant as many as a fifth or sixth appeared to be in this state." Darwin also expressly states that contabescens anthers occurred as a result of illegitimate pollination in *Primula sinensis* and *Primula veris*.

We can hardly fail to agree that Darwin was right when he said that defective pollen could arise within a single well-circumscribed species as a result of illegitimate pollination. We now know, thanks to the investigations of Bateson and Gregory, that heterostylism in *Primula* is inherited as a simple Mendelian character. Legitimate pollination maintains heterozygosis: illegitimate pollination, on the contrary, leads to the extraction of dominants which, in a sense, are genetically "purer" than the heterozygotes. Nevertheless, they may show, apparently as a result of their increased "purity," the very character of the pollen which Bateson and Jeffrey consider a sign of hybridity. A further interesting fact relating to *Primula* is as follows: strains of *P. sinensis* sometimes throw a mutation in which style and anthers are of the same length. This form, called equal-styled by Darwin, has been shown to be recessive to the long-styled form, which, in turn, is recessive to the short-styled form. Darwin cultivated several equal-styled races. He tells us that "my son, Mr. W. E. Darwin, . . . examined pollen from two equal-styled plants which he procured at Southampton; and in both the grains differed extremely in size, a large number being small and shrivelled, whilst many were fully as large as those of the short-styled form and rather more globular . . . The vast number of the small and shrivelled grains in the above two cases explains the fact that though equal styled plants are usually fertile in a high degree, yet some yield few seeds." Darwin tells us that his equal-styled races came true from seed, as, being extreme recessives, they must of course have done. Again we have a case of great genetic purity in association with defective pollen. There is no evidence, according to Bateson, that *Primula sinensis* has ever been hybridized. It seems to be one of the few cultivated plants in which great diversity has come about without any admixture with other species, although its purity is not as well attested as that of the sweet pea.

Examples might be multiplied indefinitely which show that defective pollen is as likely to indicate mutation as hybridization. In fact, I believe that it may be laid down as a rule that both processes are generally characterized by pollen sterility. With this conclusion in mind we may judge the mutation theory with a better chance of arriving at an unbiased decision.

Why is it that the polymorphic groups in which mutation is taking place, or supposed to be taking place, all show such undoubted evi-

dence of the prevalence of hybridization? The answer is simple. The mutations, as a rule, are closely enough related to their parent species so that they hybridize readily with them. By hybridization the effect of a single mutation may be widely extended, for there is no experimental evidence that mutations can be "swamped out" by hybridization if otherwise fitted to survive. In groups which have perhaps long since passed their zenith and are now represented by a few very unlike species hybridization cannot readily take place because the species which remain are too unlike to hybridize. Mutation and hybridization are usually associated with one another, and I do not see how we can escape the conclusion that hybridization is subsidiary to mutation rather than mutation to hybridization. Both processes are simultaneously concerned in the evolution of such polymorphic genera as *Oenothera*, *Rubus*, *Crataegus* and *Viola*.

In the beginning of this paper three aims of the experimental geneticist were stated. The first, as we have seen, has been attained with fair success. The origin of many spontaneous variations has been observed, and their genetic and systematic characters have been studied. The second aim was to determine the causes of mutability, so as to be able to produce the condition at will. Mutations have indeed been produced experimentally in the case of the bivalent moss varieties which have already been referred to, but other work along this line has been unsuccessful. It is impossible to view as conclusive the experiments of MacDougal in which genetic variation is supposed to have been induced by the injection of various solutions into the ovaries of *Oenothera* (*Raimannia*) *odorata* and a species called "*Oenothera biennis*." Since MacDougal's views have had such wide publicity it may be well to summarize briefly the experimental evidence which he has brought forward. One mutation is said to have been induced in the strain called "*Oe. biennis*." This strain was started from presumably unguarded seeds of one individual mother plant, of which the first generation progeny were grown in 1904. We are nowhere given any idea how large this progeny was. Four plants were self-pollinated, and the progenies were grown from each in 1905. We are told that one progeny included 669 individuals, and that the rest were not counted. With this generation of 1905, the first to be grown from guarded seed, the injection experiments were made. An ovary was injected with 1 : 500 zinc sulphate solution. The seeds obtained gave a progeny, the size of which we are not told, in which the solitary

mutation occurred. We are not told of any check cultures of sister plants from untreated ovaries, or of any further test of the strain for mutability. MacDougal referred to this experiment in 1911 as the most conclusive which he had yet performed, and stated that the mutation had come true from seed for five generations. There is no doubt that it is a very interesting mutation, especially in view of the fact that its behavior on crossing with the parent strain resembles that of *Oe. gigas*. The evidence that it was induced by the zinc sulphate solution is, however, quite insufficient. In the case of *Raimannia odorata* 13 mutations are said to have been obtained from ovaries which were injected in 1905. We are not told whether the strain had been previously purified by self-pollination or not. One ovary, injected with 10 per cent. sugar solution, gave two mutations; another, injected with 1 : 1,000 calcium nitrate solution, gave 10 mutations; a third, after exposure to radium, gave one mutation. We are not told whether the injected ovaries were all on one plant or not. There is no record of any check experiments with untreated ovaries, nor any record of the size of any of the cultures. More injections were made in 1906, with the contradictory result that calcium nitrate, which had been so potent the year before, induced no mutations at all. Three mutations were found in the progeny from "capsules" treated with 1 : 2,000 zinc sulphate, and also "other combinations" which were not followed in subsequent years. We are told, moreover, that the effect of the first injections with calcium nitrate persisted in following generations, for normal plants belonging to the strain from the ovary which was treated in 1905 with calcium nitrate gave rise to the same mutation which was supposed to have been induced by the chemical treatment. It seems to me that these facts can only mean that the strain used by MacDougal was in a highly mutable condition and that the experiments were not properly checked. It is especially noteworthy that Compton, also working with *Raimannia odorata*, has been unable to induce any mutations by the use of MacDougal's method. His strain was doubtless a non-mutable one. It is much to be hoped that more decisive results will attend future work along this line, and that whoever undertakes such experiments will adequately check them.

The third aim of the experimentalist was to provide a basis from which the systematist might determine deductively the genetic relationship of organisms. It must be admitted that except in the case of Mendelian varieties little progress has been made. Nevertheless

certain negative conclusions which may be helpful may be made. In the first place, we must conclude that the degree of sterility which follows hybridization can not be used as an index of relationship. The swarm of 200 elementary species which are included under *Erophila verna* all differ from one another in relatively trivial characters. Rosen has shown that some of the hybrids among them are fully sterile and only one pair of the species which he tried gave fully fertile hybrids. Yet we can not doubt that all are closely related genetically. In the *Onagra* group of *Oenothera* there are many species which cross more readily with *Oenothera Lamarckiana* than the latter does with its mutation *Oe. gigas*. On the whole, we may say that among closely related forms neither interspecific sterility nor the lack of it is a true guide to the degree of relationship.

The origin of a form by hybridization should not be inferred from likenesses to both of the supposed parents, nor should a high degree of sterility be interpreted as a sign of hybridization. On the other hand, true interspecific hybrids in some cases show almost no influence of one or the other parent, and are as fertile as, or more fertile than, either parent.

The extreme morphological dissimilarity between some mutations and their parent species must teach us that little reliance can be placed upon the guesses of systematists regarding relationships in polymorphic groups. The herbarium botanist should fully realize the fact that his schemes of classification, in *Rubus* and *Crataegus* for example, are probably entirely artificial and do not represent natural relationships at all. Very important systematic characters may originate repeatedly and independently in unrelated lines of descent.

There is no test of what constitutes a species, except that it shall reproduce itself from generation to generation. Systematists should have a pragmatic attitude in describing species. Subdivision should extend as far as any one finds necessary. The geneticist needs to have definite designations for much smaller groups than the ecologist or morphologist is likely to be interested in. The makers of manuals should therefore endeavor to produce books which will supply the needs of either class of workers without misrepresenting facts. If it is necessary to have a more simple treatment of *Crataegus* than that of Sargent, for example, it may be done in such a way as not to discredit a large amount of careful work. The synonymy which is so conspicuous a feature of systematic work should be given a different significance.

There should be some discrimination between true synonyms, which are names applicable to the same identical organism, and the names of distinct units which it may not seem desirable to differentiate in a popular manual.

The problem of genetic relationships is the greatest problem of biology. Only repeated attacks, from every side, will solve it. It is no reason for pessimism that results come slowly. Centuries may pass before the greater trends of evolution will be understood. In the meantime we must not scorn our advances, even though they seem slight.

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